Nitrate and Ammonia in Rumen of Steers Fed Millet

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Forages with higher than usual levels of nitrate vary in their toxicity to animals (Chapman et al., 1963; Allaway et al., 1963). This indicates that other factors in the diet may affect the rate at which nitrate and its decomposition products are metabolized. Molybdenum has been found to be part of nitrate reductase in microorganisms (Nicholas and Nason, 1954) and higher plants (Spencer and Wood, 1954). Nicholas (1959) showed that molybdenum, copper, iron, magnesium, and manganese were required for the reduction of nitrate to ammonia in *Neurospora*. Lewis (1951) and Barnett and Bowman (1957) observed that rumen microorganisms reduced nitrate to nitrite, and thence to ammonia. Tillman et al. (1965) found that molybdenum increased nitrate reduction and copper and iron increased nitrite reduction in the rumen of sheep fed purified diets.

The present investigation was designed to grow millet with high levels of nitrate with increased concentrations of copper and molybdenum through fertilization, and demonstrate how these two elements influence the rate of decomposition of the nitrate and concentration of ammonia in the rumen fluid of steers.

Acknowledgment

This paper is from a thesis submitted by the senior author to the Graduate School, University of Florida, in partial fulfillment of the requirements for the Ph.D. degree. He was a recipient of a National Institutes of Health Training Grant (5T1 GM-377 NTS) during his graduate studies. The writers wish to express appreciation to J. E. Moore, C. J. Wilcox, J. P. Boggs, G. K. Davis, and H. L. Chapman, Jr., for assistance and advice during this study.

Experimental Procedure

Gahi millet was planted April 6, on dominantly Leon fine sand and Orlando fine sand. The land had been cleared 5 years previously and no trace element fertilizer previously applied. At planting time 830 kg 10-4.4-8.3 NPK fertilizer per hectare was applied. All plots were top dressed with 112 kg nitrogen as liquid ammonia on April 28 and 494 kg ammonium nitrate per hectare on May 12, for a total of 368 kg N per hectare. The land was divided into four plots with the following minor element treatments, as follows: plot A, none; plot B, 31 kg $CuSO_4 \cdot 5H_2O$ per hectare at planting; plot C, 2.5 kg $Na_2Mo_4 \cdot 2H_2O$ per hectare at planting; plot D, combination of both B and C treatments. The millet was harvested with a forage harvester on May 21, and the chopped forage put in large loose-weave burlap bags and dried at a commercial drying plant. Nitrate was determined by the method of Woolley et al. (1960), molybdenum and copper using methods of Sandell (1959) and sulfate by the procedure of Steinbergs (1953). Nitrogen was determined by the Kjeldahl procedure (A.O.A.C., 1960). The four millet hays were fed to four rumen-fistulated yearling Hereford steers in a 4 x 4 Latin Square design. The steers were fed in individual pens with an exercise yard. The hay was offered to the steers for 1 hr. at 8:00 AM and 1 hr at 4:00 PM to have a maximum rate of intake during feeding time. After a preliminary period of 14 days, immersible lift-type rumen pumps were installed in the fistulas at 5:00 PM and the drinking water was removed. The use of the pumps gave rumen fluid samples which had been strained through 100-mesh nylon cloth. The following morning, rumen samples were obtained, after which the steers were allowed to eat and drink. After 1 hr the feed and water were removed, and 11 rumen samples were obtained at intervals over an 8-hr period after the millet was offered. The pH of the rumen samples was determined approximately 1 min. after removal from the steer. Samples were preserved by adding concentrated hydrochloric acid to pH 1 to 1.5, frozen and analyzed within a few days for ammonia nitrogen (NH_3-N) by the microdiffusion method of Conway (1957). They were then filtered through analytical filter aid with vacuum and nitrate determined by the method of Woolley et al. (1960).

RESULTS AND DISCUSSION

The nitrate, protein, molybdenum, copper, and sulfate composition of the millet is presented in Table 1. The observations made for the effect of molybdenum and copper levels in millet on nitrate concentration in the strained rumen fluid of the steers are graphed in Fig. 1. Each value plotted in Fig. 1 represents an

TABLE 1
Composition of millet, dry weight basis

Treatment Mo	Treatment Cu	NO_3	Kjeldahl N × 6.25	Mo	Cu	Inorganic SO_4
		%	%	ppm	ppm	%
0	0	0.75	11.5	0.6	13.7	0.21
0	+	0.39	12.7	0.6	11.7	0.21
+	0	0.33	13.2	3.0	9.2	0.16
+	+	0.49	13.2	4.3	10.7	0.32

average of four animals. Prior to feeding, the concentrations of nitrate in the rumen fluid were quite uniform among the treatment groups and being from 4.2, 3.0, 3.8 to 4.5 mg per liter for the control, copper, molybdenum, and copper plus molybdenum treatment groups, respectively. Because of variation in the concentration of nitrate in the millet of the different molybdenum and copper treatments and the variation in consumption of the millet, the total intake of nitrate during the 1-hr feeding period was 38, 21, 9, and 19 gm for the control, copper, molybdenum, and copper

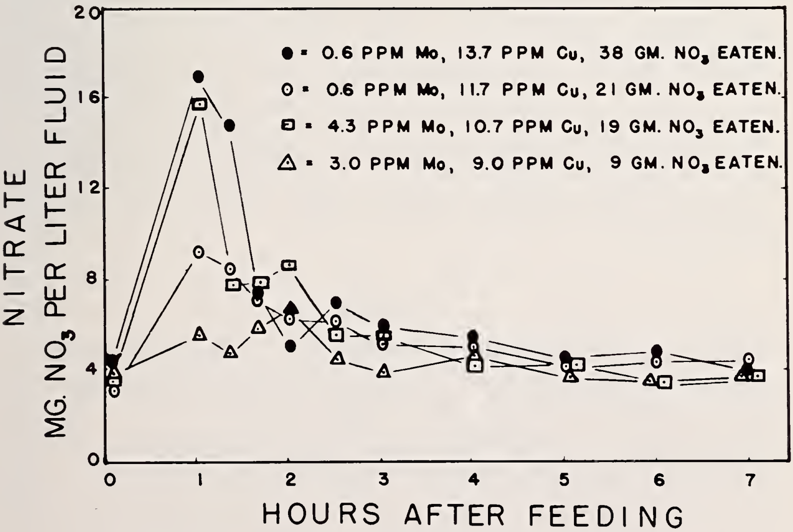

Fig. 1. Nitrate disappearance in steer rumen when fed in millet with different levels of Mo and Cu.

plus molybdenum groups, respectively. Immediately after the 1-hr feeding period, the concentration of nitrate in the rumen fluid was 16.7, 9.4, 5.4, and 15.4 mg per liter 1 hr after the start of the feeding period for the above four treatment groups, respectively. At the 3-hr period corresponding concentrations were 5.7, 5.2, 3.8, and 5.6 mg nitrate per liter of rumen fluid, respectively. Subsequently, hourly values from 3 through 7 hours ranged within the limits of 3.3 to 5.3 mg nitrate per liter for the four treatment groups. This is in the range of concentration of nitrate just prior to feeding the millet. The variation in nitrate disappearance due to different molybdenum and copper levels in the millet was not significant.

The finding that equivalent amounts of nitrate disappeared from the rumen when forages of different levels of molybdenum and copper were fed was probably due to an adequate level of molybdenum present in the forage not fertilized with this element. The low level of approximately 0.6 ppm of molybdenum in the unfertilized millet was apparently sufficient for the rumen microorganisms to build an effective nitrate reducing system in the rumen of the cattle. Thus, the minimum requirement of ruminants for molybdenum for nitrate reduction was not determined. The work by Tillman et al. (1965) with synthetic diets for sheep showed that 1 ppm of added molybdenum gave a nitrate reductase response. Sheriha et al. (1962) reported that 1 per cent potassium nitrate added to a basal diet containing 0.01 ppm molybdenum did not increase the dietary molybdenum requirement of sheep. In the present study copper levels in the millet ranging from approximately 9 to 14 ppm had no effect on the rate of nitrate disappearance.

Average values for NH_3-N in the rumen fluid of the steers obtained (1) after an 11-hr fast and just prior to the morning feeding, and (2) following a 1-hr period of feeding millet exposed to the four molybdenum and copper treatments are presented in Fig. 2. Eleven samplings were made between 1 and 8 hr after feeding began. A maximum NH_3-N level of approximately 140-160 mg per liter was reached at the 90-min interval. The NH_3-N then decreased steadily until the pre-feeding fasting level was reached at approximately 5 hr after feeding. During the next 3 hr values were slightly lower than those observed just prior to feeding. This

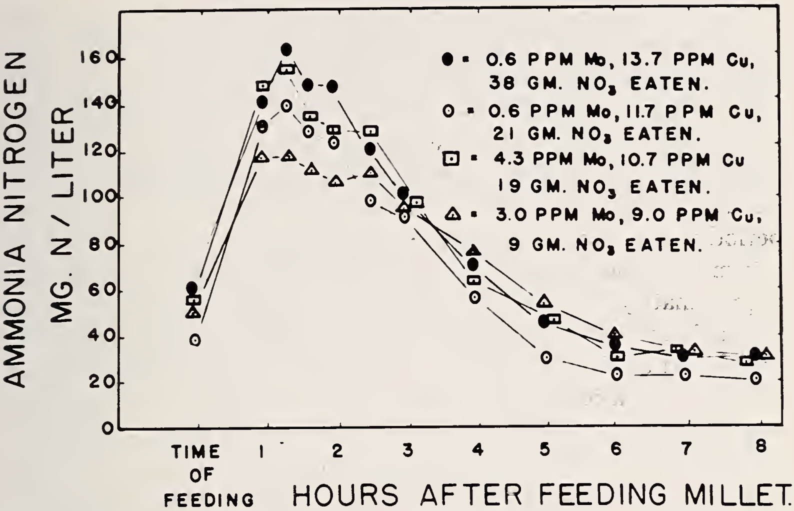

Fig. 2. Ammonia nitrogen in the rumen fluid of steers prior to and following a 1-hr. period of feeding millet containing different levels of molybdenum and copper.

may have been due to a difference between daytime and night metabolic activity in the rumen. The molybdenum and copper treatments had no significant effect on the NH_3-N concentration in the rumen. The trace mineral treatments of the millet had no significant effect on the pH of the rumen fluid.

Sulfate determinations were made on the forage as it has been demonstrated to be related to molybdenum and copper in animal nutrition (Dick, 1954). The variations in the amounts of sulfate in the millet had no influence on the factors observed in the present study.

SUMMARY

Millet was grown with high levels of nitrogen with and without molybdenum and copper fertilization. Sodium molybdate added at the rate of 2.5 kg per hectare at planting time increased the molybdenum content of the forage from approximately 0.6 to 4.3 ppm. Thirty-one kg of copper sulfate per hectare applied at planting time did not increase the copper level in the millet.

Levels of forage molybdenum ranging from 0.6 ppm to 4.3 ppm had no consistent effect on nitrate concentration in the rumen of

fistulated steers, indicating that rumen microorganisms can build an effective nitrate reducing enzyme system with as little as 0.6 ppm molybdenum in the ration. Similarly, levels of copper in the millet ranging from 9.2 to 13.7 ppm had no effect on the disappearance of nitrate. After fasting the steers overnight a maximum concentration of nitrate occurred in the rumen fluid at the end of a one-hour feeding period. Within three hours after the feeding period was ended the nitrate concentration in the rumen fluid had returned to the pre-feeding level. Just prior to feeding there were approximately 35 to 55 mg NH_3-N per liter of rumen fluid present. A maximum of approximately 110 to 160 mg NH_3-N per liter was found at 50 to 70 minutes after the 1-hr feeding period began. Within 4 hr after feeding ended the pre-feeding level of NH_3-N was observed.

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